Identifying Local Strains of *Oreochromis niloticus* That Are Adapted to Future Climate Conditions

Climate Change Adaptation: Indigenous Species Development/Experiment/13IND01PU

Emmanuel A. Frimpong[1], Stephen Amisah[2], Gifty Anane-Taabeah[1,2], Akwasi Ampofo-Yeboah[3], and Eric Hallerman1

[1]Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA
[2]Department of Fisheries and Watershed Management, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[3]Department of Fisheries and Aquatic Resources Management, University of Development Studies, Nyankpala Campus, Ghana

ABSTRACT

Climate change can severely impact the food security of tropical developing countries that rely heavily on fisheries and aquaculture for sustenance. Nile tilapia (*Oreochromis niloticus*, or tilapia) is the most widely cultured species of fish in Africa and counted on for future food security. Thus, it is important that *O. niloticus* does not succumb to climate change.

This study was conducted to synthesize information on the ambient water quality (temperature, dissolved oxygen and salinity) for *O. niloticus*, to compare wild populations in the Volta basin and the eighth generation of the selectively bred Akosombo strain from the basin used in fish farming in Ghana under current and future climate conditions, and to develop predictive models to delineate the boundaries of the species' native range to aid in identifying populations with extreme adaptations for future selective breeding in Ghana of the species for aquaculture under climate change. A combination of literature survey, field, and laboratory studies provided data for meta-analysis, growth and genetic analysis, as well as distribution models.

We found variations in water temperature along the latitudinal gradient in Ghana, and temperature was the most informative variable in terms of characterizing the adaptive range and ambient water quality for the species. However, the distribution model for *O. niloticus* did not identify maximum temperature as independently important in delineating the northern limit of the species' range in West Africa. The results of the laboratory growth studies showed no evidence of superior performance of the Akosombo strain over the wild strains under current or predicted future climatic conditions of temperature, dissolved oxygen (DO), or salinity. The results of the genetic analysis also showed that the Akosombo strain was well differentiated from all the wild populations (Aframso, Sabare and Binaba) studied. The combined results of the field, growth and genetic studies show that at least one wild population from the Oti River (Sabare) may possess the traits for superior performance under high temperature and low DO conditions. Further studies should concentrate on comparing the Sabare strain with the Akosombo strain under both lab and pond conditions and increase experimental replications to confirm the suggested differences and the heritability of those performance traits for selective breeding.

INTRODUCTION

The world's climate is changing directionally, and these changes will or are already having severe consequences for fisheries and aquaculture and food security, especially in tropical developing countries (Handisyde et al. 2005, Ficke et al. 2007, Leung and Bates 2013). Among impacts in aquatic systems expected to worsen over time include increased temperature and decreased DO, increased salinization of underground water and intrusion of salt water from sea level rise, and increased incidence of disease outbreaks in culture systems (Handisyde et al. 2005, Ficke et al. 2007, Williams and Rota 2010, Leung and Bates 2013). Recommended solutions emphasize adaptations and interventions that are based as much as possible on local practices and traditions, e.g., developing tolerant strains of existing aquaculture species and enhancing the resilience of communities, ecosystems, and traditional culture techniques (Williams and Rota 2010).

Nile tilapia (*O. niloticus*) is the most widely cultured species of fish in Africa and counted on for future food security. If this species succumbs to climate change, it will be a devastating blow to aquaculture development on the continent. Traditionally, strain selection and breeding has targeted a few traits, primarily fast growth (e.g., GIFT and its derivatives) and other desirable traits are secondary. However, planning for climate change presents a different challenge; cultured strains have to survive the climate and then grow. Temperature and DO tolerance have not been primary traits for selection because the species is considered tolerant. There has been research on hybridization of *O. niloticus* with its salinity-tolerant confamiliar species, e.g., *O. mossambicus* and *Sarotherodon galilaeus* (Kamal and Mair 2005, Yan and Wang 2010) and introduction of marine species' DNA into gonads of *O. niloticus* (El-Zaeem et al. 2011), the goal being to develop more strains that can survive and grow better in high salinity (El-Sayed 2006). The selection and development of better strains locally is encouraged over introduction of strains developed in other parts of the world. On this principle, the Volta strain of *O. niloticus* has been developed in Ghana and there are wide reports of promising performance (Dewedar 2013).

Optimal temperature for survival and growth of *O. niloticus* has been studied under a variety of conditions. Most studies found an optimum of 26-30^{o}C for growth, FCR and/or survival (Likongwe et al. 1996, Al-Asgah and Ali 1997, Baras et al. 2001, Azaza et al. 2008, El-Sayed and Kawanna 2008, Drummond et al. 2009, Xie et al. 2011). Perhaps, more intriguing is the variation observed in the optimum and the reduced growth and increased mortality past the optimum, well before the upper lethal temperature (preceding references). Strain and acclimation conditions account for some observed variation, but what has not been studied well is whether optimum and lethal temperatures vary within the species. These studies often assume implicitly that the physiological adaptations of the species are the same for all populations and individuals, and that phenotypic plasticity explains observed variation in tolerances. But it is well-known in fishes that geographic cline in traits occur. For example, the mummichog *Fundulus heteroclitus* distributed along the east coast of the United States shows a latitudinal cline in temperature and oxygen use adaptation, with underlying genotypic variation in the allelic isozymes of lactate dehydrogenase (LDH-B) that affect ATP levels (Place 1983). Individual variation in salt tolerance in *O. niloticus* has also been studied at the molecular level, although not in the context of latitudinal clines (Rengmark et al. 2007).

Sub-Saharan Africa pond aquaculture is heavily dependent on natural ambient DO as aeration is rare to nonexistent and mostly unnecessary at moderate stocking densities. Tolerance of *O. niloticus* to low DO should be understood in the context of minimum DO required for acceptable survival, growth, and reproduction. Under recirculating conditions, *O. aureus* had better FCR with intermediate (3.75±0.12 ppm), compared to low and high DOs (Papoutsoglou and Tziha 1996). DO levels do not only influence feed intake in *O. niloticus*, but it also affects growth, size at maturity, gonadosomatic index (GSI), egg size, and absolute fecundity (Kolding et al. 2008; Tran-Duy et al. 2008). There are well known dependencies among temperature and solubility of salt and oxygen, and plastic response of fish to one physicochemical variable also depends on the level of other variables, in addition to interaction of

genetics with environmental factors (Charo-Karisa et al. 2006; Schofield et al. 2011). A quick search of Web of Science and Aquatic Sciences and Fisheries Abstracts databases for the period 1970-2012 revealed more than 1,100 peer-reviewed publications on *O. niloticus* and and confamiliar species that focus on some aspect of growth. However, on close examination, West Africa native strains of *O. niloticus* are under-represented in these studies. Most studies are from Egypt or otherwise from outside of the continent. Identification of better-adapted populations of *O. niloticus*, and degree of adaptation to temperature, DO, and salinity will require synthesis of existing knowledge on the species, a combination of field and laboratory studies, including basic genetic descriptions, and linkage of distribution with biophysical data.

Species exhibit their most extreme adaptations at the tail ends of their range and in response to environmental gradients (e.g., Place 1983). In West Africa, natural climate varies from humid forest to dry savanna and desert as you move from the coast (low latitude) to interior (high latitude). The Volta basin, which spans the entire length of Ghana into Burkina Faso, aligns with this gradient. Reported occurrences (Paugy et al. 2003) indicate that *O. niloticus* range crosses much of the climate gradient. Populations in hot, drought-prone areas are predictably better adapted to high temperature, low DO, and high salinity. The salinity prediction is less intuitive but not when you consider that aquatic systems subject to high evaporation tend to have higher salinities, as drying concentrates dissolved solids. Thus, the predicted climate change scenarios in the southern parts of Ghana where most aquaculture is concentrated is very similar to current conditions in the north, near the upper limit of known *O. niloticus* West African range. A more accurate zoogeographic model for *O. niloticus* would be useful as a tool to identify the best adapted populations and also delineate the species' range from the numerous cichlid species (more than 40 in West Africa, Paugy et al. 2003) that are undoubtedly confused with *O. niloticus*, especially by small-scale farmers who still rely significantly on wild brood and seed to stock their ponds. Identifying these populations and their adaptations will guide future breeding programs that will have to consider climate, and take advantage of individual variation within populations to select for desired traits in addition to fast growth.

OBJECTIVES

- Conduct a comprehensive review, a meta-analysis, and synthesis of the peer-reviewed literature on *Oreochromis niloticus*, with respect to various strains and their adaptive range for temperature, dissolved oxygen (DO), and salinity;
- Conduct laboratory experiments to test the tolerance of the Volta strain and three wild populations of *O. niloticus* to increased temperature, decreased DO, and increased salinity;
- Determine size distribution, sex ratios, and length-fecundity relationships and characterize ambient water-quality (temperature, DO, salinity) of *O. niloticus* in its native habitat along the latitudinal gradient from southern to northern Ghana in the Volta basin;
- Genetically characterize wild populations of *O. niloticus* along the latitudinal gradient of the Volta Lake; and
- Develop a predictive distribution model (i.e., zoogeography) for *O. niloticus* in West Africa and accurately delineate the extremes and boundaries of the species' native range.

Hypotheses for experimental studies

***Hypothesis 1*:** The Volta/Akosombo strain of *O. niloticus* grows faster compared to the average wild strain in Ghana under current climate conditions, but the Volta strain is less tolerant of high temperature, low DO, and high salinity.

***Hypothesis 2*:** Northern populations of *O. niloticus* in West Africa are more tolerant of high temperature, low dissolved oxygen, and high salinity than southern populations; and the northern populations have a higher optimum temperature for growth.

MATERIALS AND METHODS

Study area. The study was conducted in the Volta basin of Ghana with field sites at Aframso in the Afram sub-basin, Sabare in the Oti sub-basin, and Binaba in the White sub-basin of the Volta (Figure 1). The distribution model covered the entire West Africa range of *O. niloticus*.

Figure 1: Field sites (Source of base map: www.mapsofworld.com).

Meta-analysis. We surveyed the primary literature for studies that investigated the effect of temperature, DO and salinity on the growth of Nile tilapia, *O. niloticus*, for a meta-analysis. An initial search for papers with the keywords including "growth" and "tilapia" yielded >1,100 hits in Web of Science, of which full text were accessible for 762. Of these, 57 papers were identified to be relevant to the objectives of this study and only 19 provided data that could be reanalyzed (Appendix A). Eight studies investigated temperature effects, nine studies investigated salinity effects and three studies investigated DO effects on growth. We observed almost no consideration of interaction of the three factors among studies, with only one study investigating the combined effect of temperature and salinity on growth on Nile tilapia. We excluded studies that focused only on the egg to fry stages of growth in Nile tilapia, since sample sizes were too small to include analysis of ontogenetic change in tolerance to temperature, DO, and salinity. When data were extracted and catalogued in Excel, the 19 studies together provide 140 rows of data, including direct measurements (or data to calculate) specific growth rate (SGR), length of study, initial and final sizes of fish used in the study, and the specific experimental levels of temperature, DO, and salinity. Although we recoded the tear of study, strain/origin, culture system and other experimental factors, these pieces of information were not available for most studies and samples size did not permit incorporation to a large number of factors in the meta-analysis.

To use an approach that accounted for between study variability without prohibitive cost in degrees of freedom, we first ran a regression with "study" as the predictor of SGR and saved the residual SGR for subsequent analysis. Thus, the models comparing temperature, DO, and salinity effects on SGR each used the residuals. Based on visual inspection of the plot of SGR against each of the three factors, we fit a quadratic regression between temperature and SGR and linear relationships for DO and salinity.

Laboratory challenge and growth experiment. For the laboratory growth experiments, we collected wild broodstock in January 2015 from sampling the Afram River, White Volta and River Oti in Aframso, Binaba and Sabare townships respectively. Broodstock were kept in hapas mounted in a 200 m^2 pond at Kwame Nkrumah University of Science and Technology (KNUST) fish farm and fed high-protein diets for six months. This period served as acclimation for the fish and provided sufficient time for spawning and growth of juveniles of for the laboratory experiment. All fish were uniquely tagged with elastomer tags at the beginning of the experiment to aid in individual monitoring of fish. We used a factorial design with three factors (temperature, DO, and salinity) with two levels of each factor (low and high) and three replicates, for a total of 24 experimental units (aquarium tanks), each with at least one individual from each river (site, population, or "strain" hereafter) and the and the Akosombo strain. Fingerlings of the Akosombo strain (eighth generation) was obtained from the Pilot Aquaculture Center (PAC) of the Fisheries Commission of Ghana and kept in the same pond with the other strains for acclimation.

High water temperature was set at 33ºC with individual aquarium heaters whereas the room temperature (about 24ºC) was default for low temperature. High DO was obtained by constant aeration using multiple air stones, in addition to aquarium filters deployed for all experimental units. High salinity was achieved through gradual addition of crude-salt concentrate to the tanks over three days to achieve desired salinity of 15 ppt. A pretest was run for two months prior to the start of the experiment to refine the design and ensure that the desired factor settings were achievable in the experiment. For the main experiment, five uniquely tagged fish (majority between 5g and 8g) were randomly assigned to the treatment tanks, one from each of the three study sites including an extra site in Binaba. The Akosombo strain of *O. niloticus* served as control. We replaced fish that died in the first three days after stocking, after which no further replacements occurred. Fish were fed to satiation 3–4 times daily with a 48% protein commercial feed for the duration of the experiment and the daily feed given was recorded per tank. Temperature, DO, salinity, pH and conductivity were measured daily between 10:00 and 12:00 h with the Hanna multiparameter meter (HI 9828). Ammonia, nitrite and nitrate were measured three times during the experiment with the API Freshwater Master Test Kit. Final fish weights were recorded after 17 days. Growth was expressed in terms of percentage daily weight gain or specific growth rate [(Ln final weight - Ln initial weight) / time × 100]. A general linear model with two-factor interactions was used to analyze the data statistically, in addition to graphical analyses.

Field studies. Fish sampling for the life history studies was conducted with the help of local fishermen between November 2014 and July 2015. Total fish catches varied by month across all three sites, with some sites recording no catches due to high water levels precluding sampling either by seining or cast nets. Fish were measured for total (TL) and standard lengths (SL) to the nearest 1 mm and weighed (total weight) to the nearest 0.1g. Fish for fecundity studies were fixed in 10% formalin solution. Preserved fish were washed thoroughly and stored permanently in 70% ethanol after three days for further analysis. Sex determination was done by physical examination of gonads and fish were described as females, males and juveniles whose sexes were indeterminate. Lengths and weights after preservation were also measured. Fish were dissected to examine gonads. Where present in females, a subsample of eggs were carefully detached from gonads, counted and weighed. Where eggs were not matured, the entire gonad was weighed. Total egg count per female (fecundity) was estimated as weight of gonads multiplied by the number of eggs in the subsample divided by the weight of the subsample. The sexual proportion of males to females was obtained. Females were considered matured if they had eggs or visibly matured gonads. The mean length at sexual maturity for the female across sites was calculated as the length at which 50 percent of all individual become sexually mature, using a logistic regression of length on maturity status.

Water quality was measured monthly at the three sites between August 2014 to July 2015. However, due to logistical constraints, water quality was not measured in December 2014 and May 2015 for Binaba and Sabare, as well as March 2015 for Aframso. The water-quality variables measured each sampling month included temperature, DO, salinity, pH, and conductivity. All water-quality variables were measured with

the Hanna multiparameter probe (HI 9828). When necessary, water samples were sent to the laboratory and the API Freshwater Master Test Kit was used to confirm some of the results obtained with the probe. Differences in average monthly water quality variables were tested with a one-way analysis of variance (ANOVA), with statistical significance set at $p \leq 0.05$.

Genetic characterization. *O. niloticus* fin clips were obtained from fish sampled at five sites in the Volta River system (Sabare on the Oti River, two sites near Binaba on the White Volta River, Aframso on the Afram River, and the widely cultured Akosombo strain from the Fisheries Commission's Pilot Aquaculture Center (PAC) in Kumasi). DNA was isolated from a sample of 30 individuals from each of the five populations. DNA was purified using a commercial kit (PureGene, Gentra Systems), and DNA concentration was quantified using a Nanodrop Lite spectrophotometer. Using polymerase chain reaction (PCR), we screened allelic variation at five microsatellite DNA marker loci for Nile tilapia (*UNH 130, UNH180, UNH858, UNH925*, and *UNH934*) linked to growth rate in other Nile tilapia stocks (Streelman and Kocher 2002, Cnaani et al. 2003). Sequence information for primers for amplifying these microsatellite loci was obtained from GenBank (www.ncbi.nlm.nih.gov/genebank/). Microsatellite amplicon lengths were resolved on an Applied Biosystems (ABI) 3100 automated DNA sequencer at the Virginia Bioinformatics Institute and scored using GeneMarker version 2.6.4. Allele frequencies, expected and observed heterozygosities, pairwise F_{ST} and analysis of molecular variance (AMOVA) were calculated from the data using the Microsat Toolkit version 3.1.1 and Arlequin version 3.5.2.2.

Modeling distribution of *O. niloticus* in West Africa. Thirty-six known presence records were obtained from Paugy et al. (2003) and compared with distribution data available through Fishbase www.fishbase.org. We concluded that the data sources were mostly duplicated. In addition, the reported distributions had large gaps in areas of West Africa where Nile tilapia is known to occur (e.g. the sites sampled in this study). Because of the unreliability of absence records, we adopted the maximum entropy (MaxEnt) presence-only machine-learning modelling approach using MaxEnt version 3.3.3k (http://www.cs.princeton.edu/~schapire/maxent/). Environmental data, principally, a 900-m resolution digital elevation model (DEM) for West Africa was obtained from US Geological Survey (USGS; http://www.webgis.com/terraindata.html), altitude and bioclimatic grids from WorldCLIM (http://www.worldclim.org/; Hijmans et al. 2005), and year 2000 population sizes for cities in West Africa from the Harvard University Center for Geographic Analysis (http://gis.harvard.edu/services/products/harvard-africa). We used the DEM to perform a watershed delineation and delineated about 17,000 subwatersheds for West Africa with areas ranging from 250km^2–500km^2 and a few >1000km^2. The subwatersed were then used as study units for summarizing environmental variables and attributing the areas of know nile tilapia presence. Final 22 variables used in the MaxEnt modeling included altitude, stream order, population density (interpolated by ordinary Krigging), and 19 bioclimatic variables (BIO1-BIO19) including temperature and precipitation measures (see Appendix E for definition of all variables). Modeling was conducted with 70% of the 36 presence points and tested with the remaining 30%, using the Area under the Receiver-Operating-Characteristic-Curve (AUC) as a measure of model performance. Probability of tilapia presence was calculated for all nearly 17,000 subwatersheds.

RESULTS

Meta-analysis. We observed reported SGR across the 19 studies ranging between 0.002%–195%, however, when we recalculated SGR based on beginning and ending sizes reported in individual studies, SGR ranged from 0.002%–15.5%. Temperature ranged between 10°C–37°C, DO ranged between 1.33mg/L–6.08mg/L and salinity ranged between 0 ppt–2 ppt for the combined experimental range. For our analysis, we truncated the temperature range to start at 20°C to approximate tropical temperatures reflective of conditions in Ghana and other West African countries. The regression models showed significant relationships between SGR (%) across studies for temperature and DO, and no relationship

with salinity. Residual plots of SGR and temperature showed a quadratic relationship (Figure 2) and described by the equation:
Residual SGR = 30.350 + 2.140*Temp – 0.037*Temp^2; R^2 = 0.342, with the highest SGR at 29^{o}C. The relationship of SGR with DO was slightly linear positive and describes by the equation: Residual SGR = -0.5581 + 0.1638*DO; R^2 = 0.104 (Figure 2).

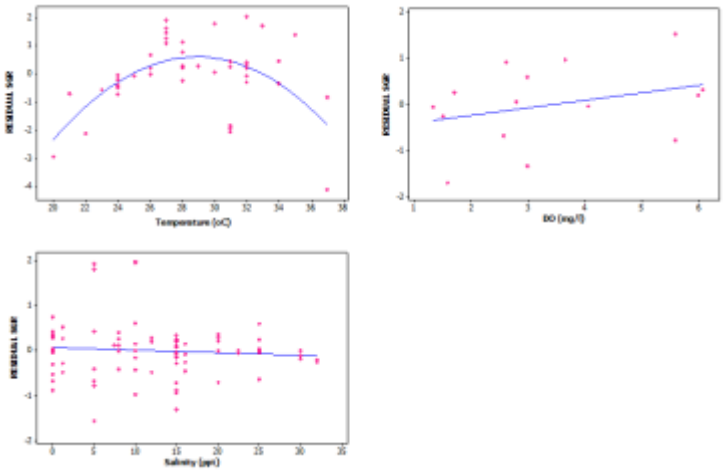


Figure 2. Plots of specific growth rate (residualized) against temperature, DO and salinity

Laboratory experiment. The mean low temperature recorded in the experimental units was 25.73^{o}C and the mean high was 32.75^{o}C. DO measurements recorded were 6.13mg/l and 7.47mg/l for the low and high levels, respectively. Salinity was 0.09 ppt for the low end and 0.15 ppt for the high end. The specific growth rate of *O. niloticus* ranged from -1.7 to 17 and showed varying sensitivity to temperature, dissolved oxygen and salinity across strains. The general linear model indicated an overall significant model, with salinity being the only significant factor at p = 0.008. In partial agreement with study hypotheses, SGR under "ideal" conditions (low temperature, high dissolved oxygen and low salinity) were relatively higher regardless of strain (Figure 3). The response to temperature by strain was mixed. The interaction plots from the general linear models showed that overall, the Sabare (SAB) strain tended to grow faster under the "ideal" conditions. Furthermore, compared to the other strains, the growth of the Sabare strain appeared to be insensitive to temperature within the range studied in this experiment, evidenced by comparable specific growth rates under both low and high temperature conditions (Figure 3). The Binaba (BIN) and Akosombo (PAC) strains showed relatively higher growth rates in relation to high temperatures while Aframso tended to be the least tolerant of high temperature (Figure 3). In terms of strain performance, the Akosombo strain was the next best after Sabare, followed closely by the Binaba strain, with the Aframso strain showing high variability in specific growth rates. Most of these trends were obvious, but not statistically significant due to small sample sizes and high variability in the response of the Aframso strain. See Appendix B for additional results.

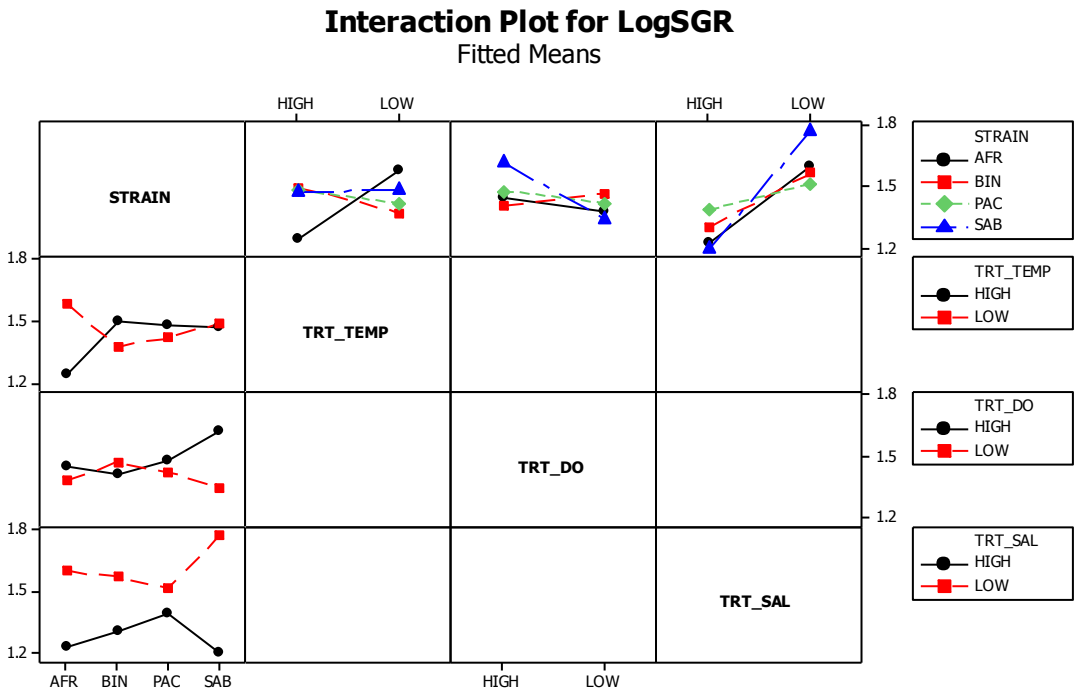


Figure 3. Interaction plots from a general linear model for specific growth rate of the different *O. niloticus* strains across the treatment factors: temperature, dissolved oxygen and salinity.

Field studies. ***Life history*** — Total length of fish captured in the field ranged from 4 cm to 25 cm with corresponding weights of 1.3g to more than 300g. The length-weight relationships observed from fish sampled at the three sites showed very little variation (Figure 4). The length-weight relation for Aframso is expressed by the regression equation: Log_{10} (Weight) = -1.786 + 3.056 Log_{10} (Length); R^2 = 0.96; N = 23. The length-weight relation for Binaba is expressed by the regression equation: Log_{10} (Weight) = -1.727 + 2.992 Log_{10} (Length); R^2 = 0.95; N = 91. The length-weight relation for Sabare is expressed by the regression equation: Log_{10} (Weight) = -1.788 + 3.054 Log_{10} (Length); R^2 = 0.99; N = 104. Overall, females dominated the samples across the three study populations (Aframso, Binaba, and Sabare). Sex ratio for Aframso was 56% female to 44% male (n = 23), Binaba recorded 66% female to 35% male (n= 97), and Sabare recorded 58% female to 42% male (n = 40). *O. niloticus* from Aframso matured early at about 13 cm, followed by Sabare and Binaba at 14.5 cm and 15 cm, respectively (Figure 5). Mature females were found in all months of the year when observations were made (December to July), with most in April and July at Binaba. Fecundity ranged between 70 to about 5200 eggs per female and had a positive linear correlation with fish size in the Sabare population. However, no clear relationship was exhibited by the Binaba population (Figure 6), and Aframso did not have sufficient mature females to estimate a length-fecundity relationship. See Appendix C for additional results.

Water quality. The water quality variables measured showed high variation but relatively minimal seasonal variation for all three sites except for a marked decrease in temperature and increase in DO in January (the cold, dry season) for the northern sites, Sabare and Binaba. However, the salinity recorded for Aframso was statistically higher compared to Sabare and Binaba across the annual cycle. Temperature was also significantly higher at Sabare on the average compared to Aframso and Binaba (Figure 7).

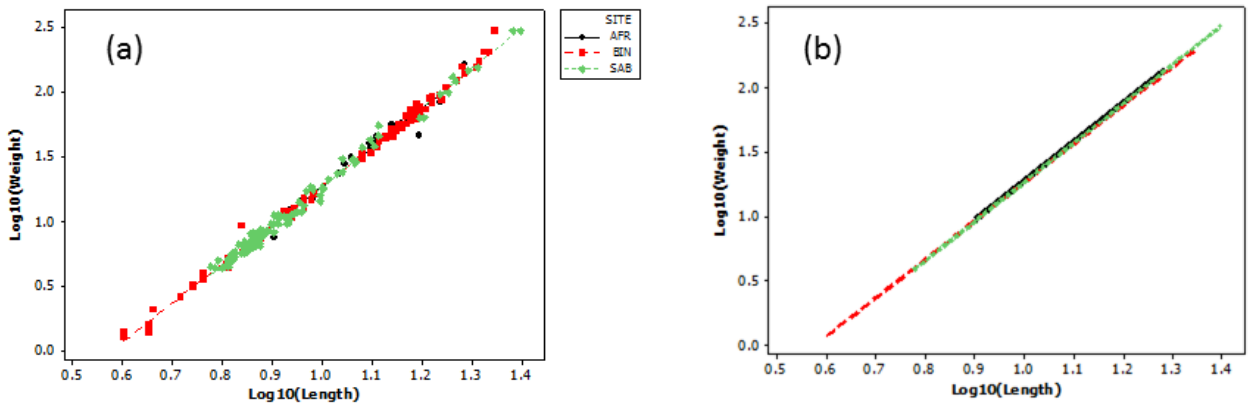


Figure 4. Length-weight relationship *O. niloticus* individuals sampled at Aframso, Binaba and Sabare (a) displayed with observed data (b) displayed without observed data.

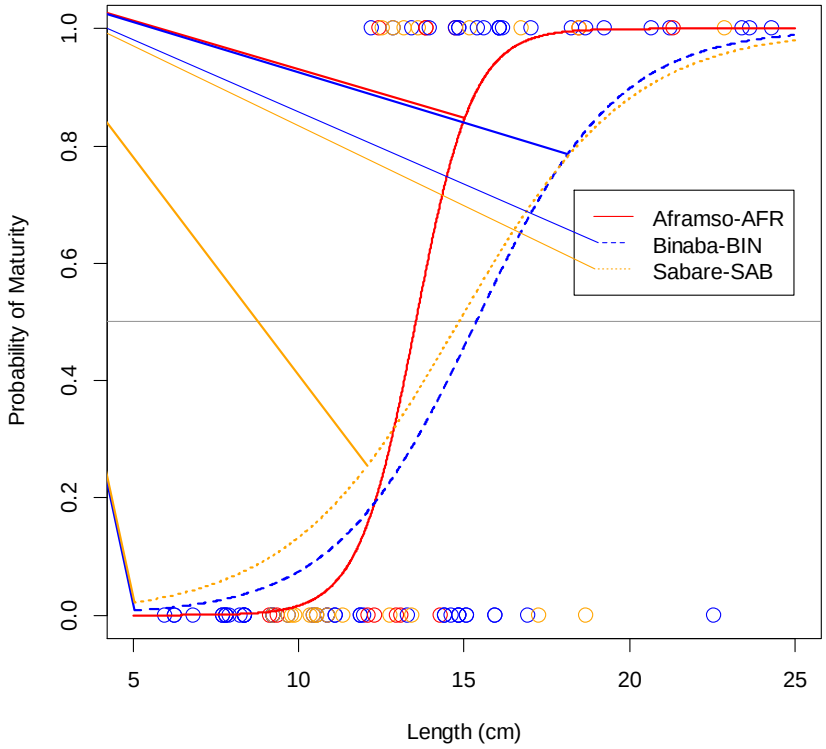


Figure 5. Logistic regression curves showing size at maturity for female *O. niloticus* sampled in Aframso, Binaba and Sabare. The black horizontal line indicates 50% maturity.

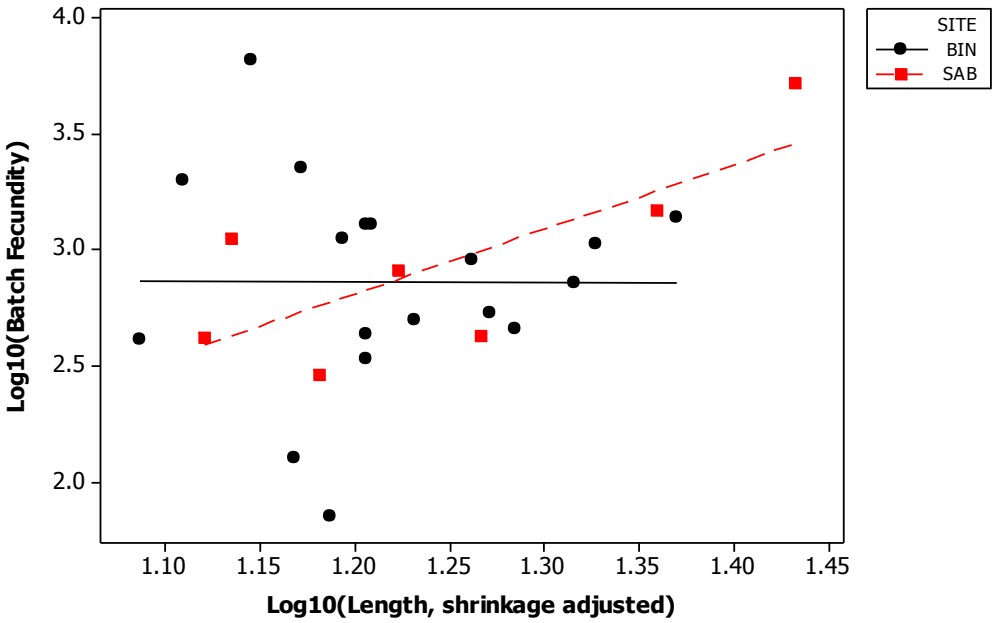


Figure 6. Fecundity versus length for females in the Binaba (BIN) and Sabare (SAB) populations

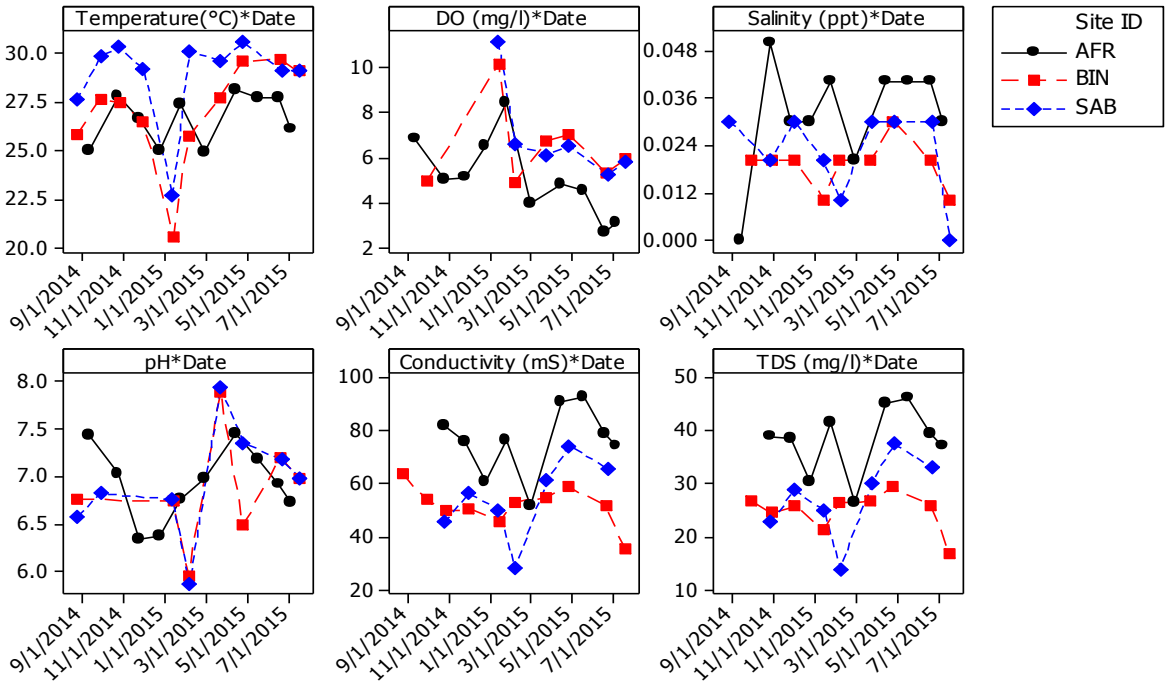


Figure 7. Water-quality variables measured in the Volta system on a monthly basis between September 2014 and July 2015

Genetic characterization of populations. The results showed that all five microsatellite DNA marker loci screened for Nile tilapia screened to be polymorphic. We found a high allelic diversity (mean ±SD of 9.4±4.9 alleles per locus per population; Figure 8) and high-expected heterozygosity (0.73 ±0.17). Results of AMOVA showed 93.3% of genetic variance within populations, 2.25% among populations within regions, and 4.47% among regional groupings of populations, a result typical for natural populations. Regional patterns of regional differentiation visualized using classical F_{ST} analysis (Wright 1965) showed considerable differentiation among the Afram and White Volta (F_{ST} = 0.049), Afram and Oti (F_{ST} = 0.027) and White Volta and Oti (F_{ST} = 0.054) populations. At the population level, the widely cultured Akosombo strain was well differentiated from the Afram (F_{ST} = 0.082), White Volta (F_{ST} = 0.080), and Oti (F_{ST} = 0.118) populations. See Appendix D for additional results.

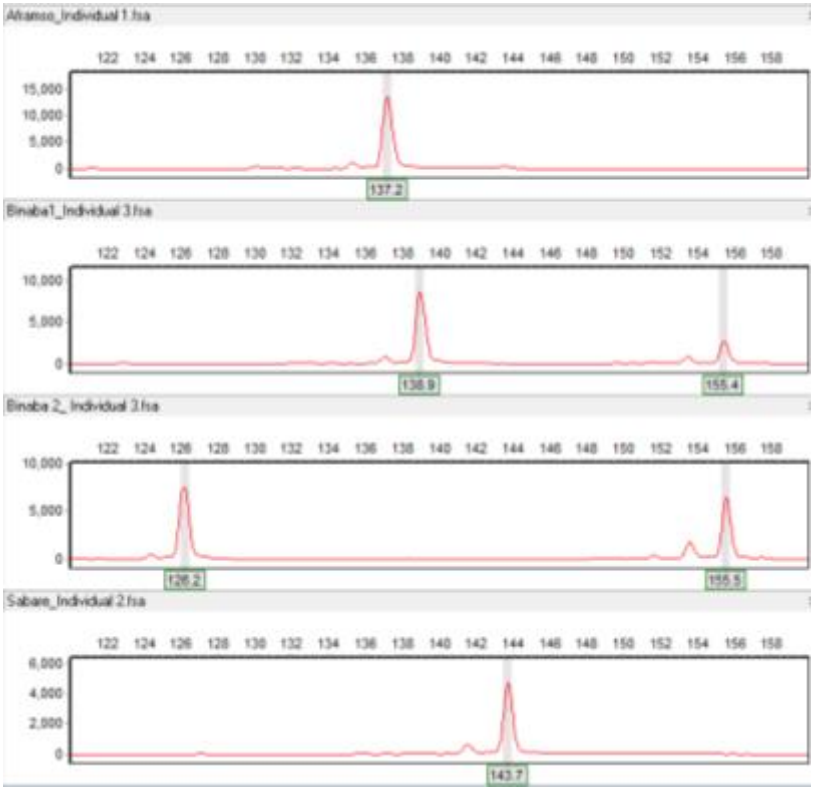


Figure 8. Output screen from Genemarker, used to interpret DNA fragment analysis results. The output above depicts allelic variation in four individuals from four populations at the UNH180 locus. Individuals with a single peak exhibit homozygosity at the locus, whereas two peaks suggest heterozygosity. The number of unique peaks are the different alleles at this locus.

Modeled distribution of *O. niloticus.* Of the 22 variables used in the MaxEent model, the important predictors of *O. niloticus* occurrence in West Africa were stream order, temperature seasonality, annual precipitation, and precipitation of the warmest quarter of the year (Figures 9 to 12); however, the raw temperature measurements by themselves were not important in predicting the species' occurrence. Altitude and population density had a negative relationship with *O. niloticus* occurrence. The resulting predictive model had a good performance, exhibited by a high area under the curve (AUC = 0.87). The model suggests that *O. niloticus* has a northerly distribution in West Africa (Figure 13), with highest probability of presence (0.4–0.91) occurring in the transition zones of the Sahel and savanna areas and furthest from the coastal and tropical forest areas. See Appendix E for additional results.

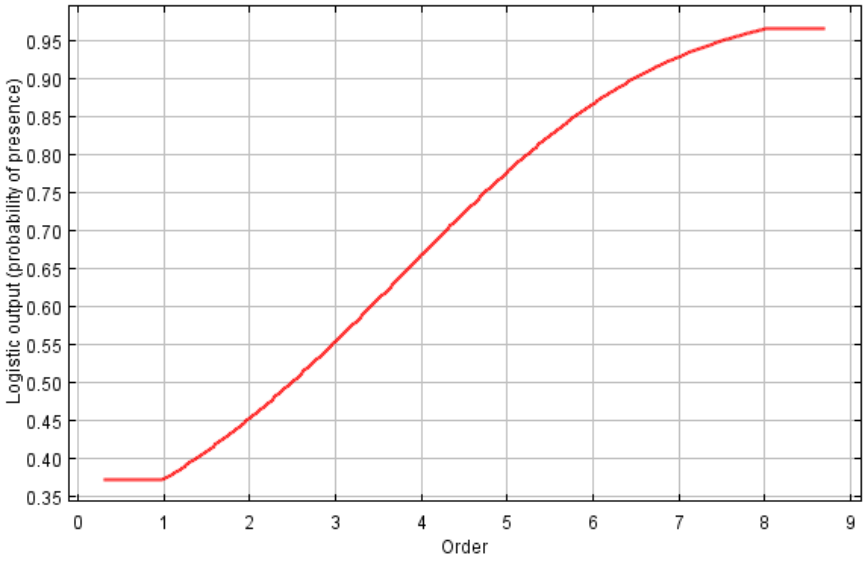


Figure 9. Response of *O. niloticus* to stream order in West Africa using raw variables uncorrected for correlation with other variables. Stream order was calculated by the Strahler method and used 250 km^2 watersheds as first order, which is an underestimation. Partial dependence plots for all variables can be found in Appendix E.

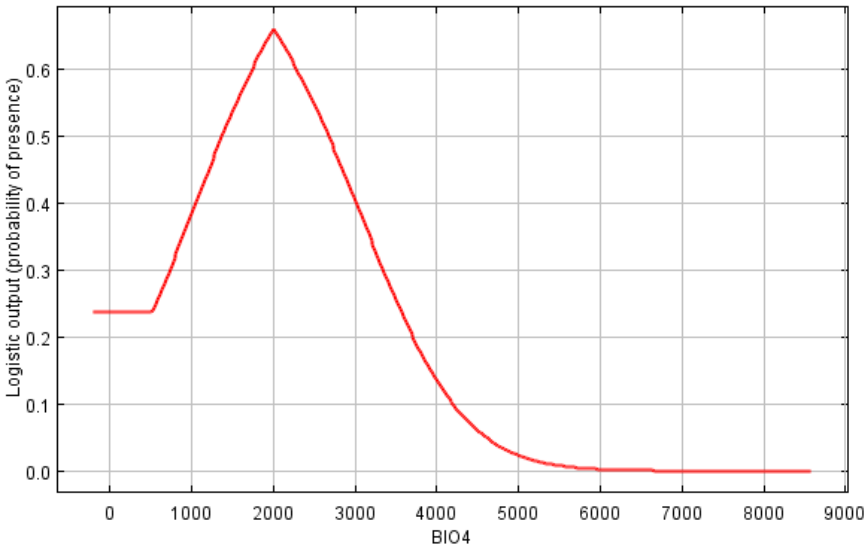


Figure 10. Response of *O. niloticus* to temperature seasonality in West Africa using raw variables uncorrected for correlation with other variables. Partial dependence plots for all variables can be found in Appendix E.

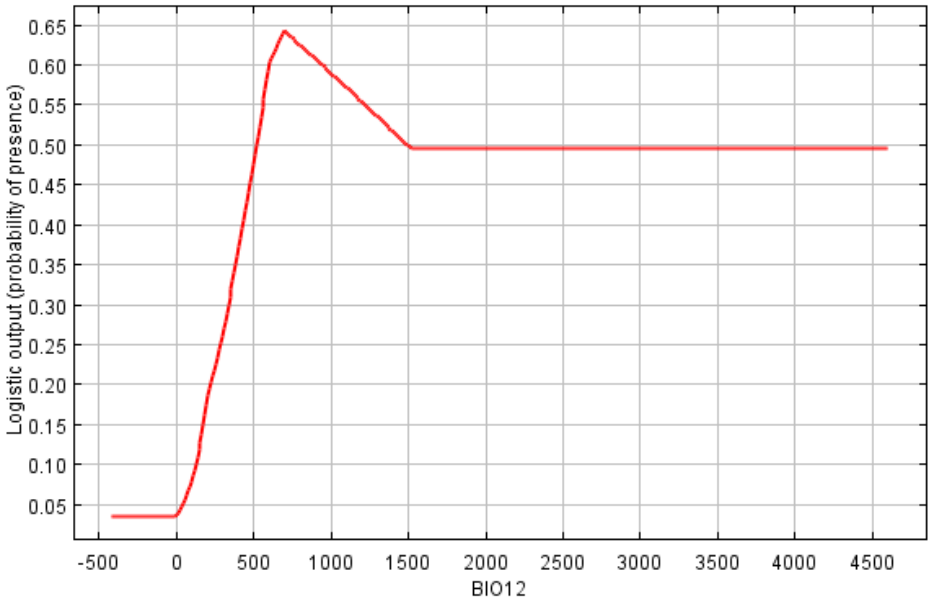


Figure 11. Response of *O. niloticus* to annual precipitation in West Africa using raw variables uncorrected for correlation with other variables. Partial dependence plots for all variables can be found in Appendix E.

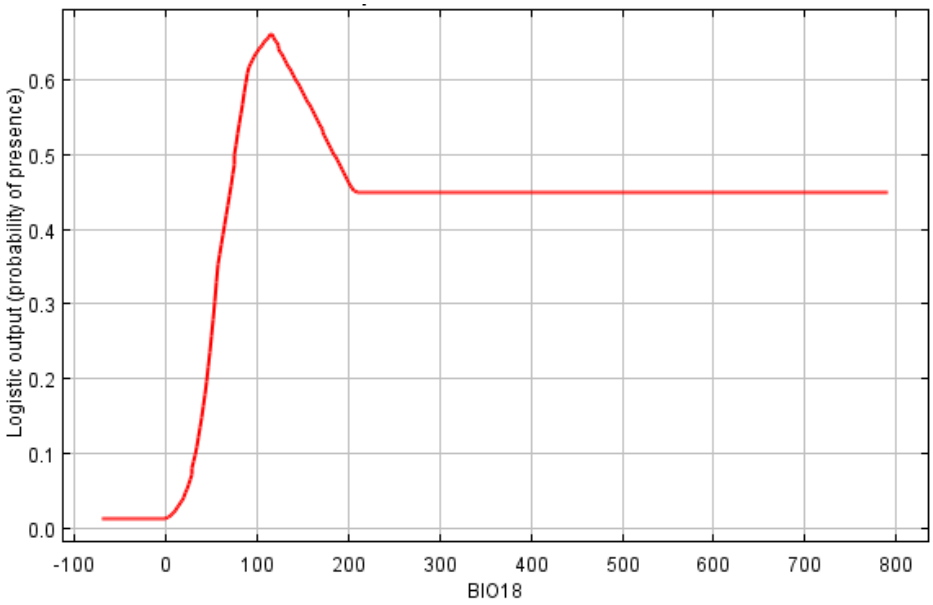


Figure 12. Response of *O. niloticus* to precipitation of warmest quarter in West Africa using raw variables uncorrected for correlation with other variables. Partial dependence plots for all variables can be found in Appendix E.

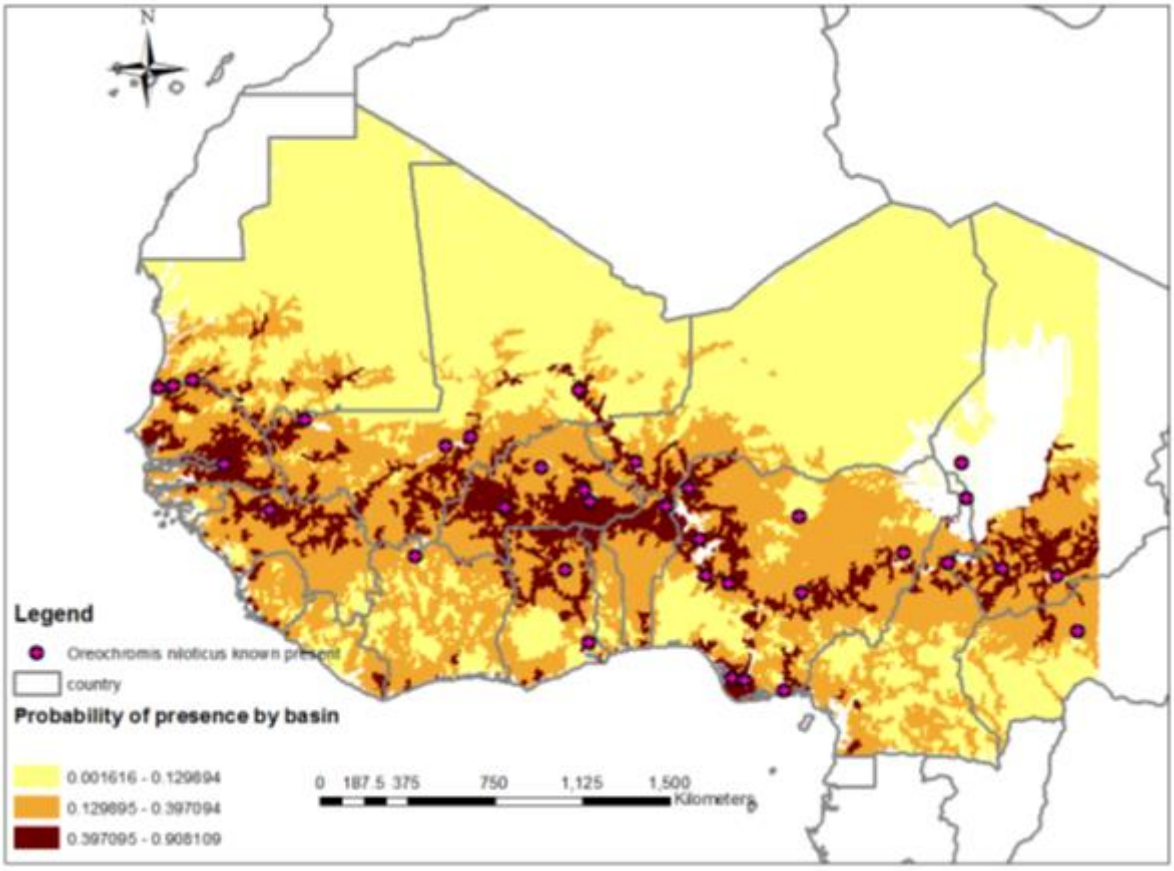


Figure 13. Predicted distribution map for *O. niloticus* in the West African subregion. Map is based on presence-only maximum entropy (MaxEnt) model based on data from Paugy et al. 2003. Brown areas have the highest probability of occurrence.

DISCUSSION

The most significant results of the meta-analysis are that the optimal temperature for growth of *O. niloticus* is about 29ºC, that growth increases linearly with DO, and salinity per se below the upper lethal limit of 32 ppt has no effect on Nile tilapia growth performance. The number of peer-reviewed studies that were available and suitable for this analysis was small and skewed toward North Africa and Asia, making the results potentially less applicable to West Africa. The DO range in the studies was limited as was the number of studies. In the context of the experimental studies section of the current report, the lack of relationship of salinity with SGR points to dependencies of growth on many additional factors, some of which were clearly not included in the meta-analysis because of limited sample sizes. In addition, the lack of studies considering interactions among temperature, DO, and salinity limit the utility of most of the studies for drawing conclusions about the optima of any variable because factor interactions appear to important. In other words, the optimum level of any factor can best be determined at the settings specified for other important factors.

The significant positive linear relationship between fecundity and size of fish observed in the Sabare population was expected. Large fish usually produce relatively more eggs and egg size is often a function of body size. A number of factors could contribute to the pattern observed between fecundity and fish size for the Binaba population. Due to variability in the monthly samples, the number of matured females across seasons was small. Even though we collected significant number of individuals for laboratory analysis ($n > 250$), the size frequencies and the number of eggs per matured females suggested that we

sampled more fish during the off peak reproduction season. It is also possible that we collected older females who had fewer eggs due to previous spawning episodes, as well as younger females who had just began spawning and had more eggs. Batch or serial spawning in *O. niloticus* is evidenced by the collection of matured females most months of the year. Thus, our estimate for fecundity is at best, batch fecundity, in most cases, which will tend to have a weak relationship with size. The similar length-weight relationships suggest that there is no significant divergence of morphology among the populations of *O. niloticus* in the Volta basin. The biased sex ratio toward females and the potential differences in size at maturity is worth further investigating with larger sample sizes and more targeted seasonal sampling. For *O. niloticus* in ponds, delayed maturation is a desirable trait.

Our first hypothesis for the laboratory experiment was that the Akosombo strain of *O. niloticus* grows faster under current climate conditions and will be less tolerant of expected future climate conditions: high temperature, low DO, and high salinity. We expected to see the Akosomso strain exhibit significantly high SGR under low temperature, high dissolved oxygen and low salinity conditions. However, no significant differences were observed between the SGR of the Akosombo strain under the low and high conditions of any of the three factors tested. These results suggest that the Akosombo strain may not necessarily be a faster growing strain even under the current climate condition, compared to the wild strains. This is an argument the fish farming community in Ghana continues to make— that the selective breeding of Nile tilapia has not yielded any improvement over the wild strain in terms of growth. On the other hand, the laboratory conditions under which growth was observed could be less than ideal for the selectively bred strain compared to the pond environment to which it has been optimized. Further investigation of the real growth performance differences between the Akosombo strain and its wild counterparts is warranted.

The growth experiment also showed temperature as an important factor differentiating the strains, partly supporting our second hypothesis that northern populations of *O. niloticus* in West Africa are more tolerant of high temperature, low DO, and high salinity than southern populations. The northern populations (Binaba and Sabare) of *O. niloticus* population in Binaba were either more tolerant or insensitive to high temperature conditions, while the southern population (Aframso) was less tolerant to high temperatures. However, the high variability in growth observed in the Aframso strain, which we attribute to small sample size caused by high mortalities during the growth experiment, precludes definitive conclusions about the Aframso population. The fact that the Akosombo strain appeared to be slightly tolerant of high temperature conditions may be attributed to the strain's adaptation to culture conditions over the many years in use in aquaculture in Ghana. More importantly, the result of the growth experiment also suggests that the Sabare strain, though wild, has a slightly better performance than the Akosombo strain even under the experimental conditions. The significantly high water temperatures recorded in Sabare in the field studies, coupled with the Sabare population's temperature insensitivity in the growth studies, suggest that the strain may perform better than the Akosombo strain under stressful conditions such as high temperatures and low dissolved oxygen. The fact that any group does better than or as well as the Akosombo strain under any of the culture conditions is a reason to continue to investigate and Akosombo breed for more tolerant and faster growing strains for Ghana fish farmers.

The results from the genetic characterization showing all five gene loci screened as polymorphic indicates that multiple alleles are responsible for growth both within and among different tilapia populations in Ghana. The high allelic diversity within and among populations is indicative that these populations have a good evolutionary potential and are currently not bottlenecked or at risk of immediate extinction. At the regional level, genetic differentiation observed among the Afram (Aframso) and White Volta (Binaba; $F_{ST} = 0.049$), Afram and Oti (Sabare; $F_{ST} = 0.027$) and White Volta and Oti ($F_{ST} = 0.054$) populations suggest that whereas both the Oti and Afram populations are quite different from the White Volta population; the Oti and Afram populations are more similar in their genetic make-up. The level of

differentiation observed between the Afram and Oti population is interesting and requires further investigation, considering that only the Afram River originates in Ghana, while both the White Volta and Oti have their sources in Burkina Faso. Perhaps, screening additional loci would help clarify the genetic differentiation between the Afram and Oti. The Akosombo strain was well differentiated from Afram (F_{ST} = 0.082), White Volta (F_{ST} = 0.080), and Oti (F_{ST} = 0.118) populations, which confirms that the Akosombo strain was probably developed from crosses involving several different strains from the Volta system. Further studies are required to ascertain this and to investigate the genetic integrity of the broodstock generally referred to as the "Akosombo" strain marketed by hatchery operators in Ghana. The apparent differentiation of the Akosombo strain from the other study strains, coupled with the results from the growth studies, warrant future heritability studies involving the Akosombo strain and the Sabare strain with a goal of developing a high performing strain adapted to stressful future climatic conditions.

The distribution model for *O. niloticus* did not identify maximum temperature as independently important in delineating the northern limit of the species' range in West Africa. The lack of true absence data limits the applicability of a distribution model as, by definition, presence-only models overestimate distribution range. Future extensions of distribution modeling of *O. niloticus* would benefit from inclusion of reliable absences and more samples from smaller water bodies which are probably undersampled by the Paugy et al. (2003) collections derived mostly from fishing (large river, reservoir, lake) data.

CONCLUSION

This study used a multifaceted approach to study the Nile tilapia populations of the Volta basin and the eighth generation of the selectively bred Akosombo strain from the basin used in fish farming in Ghana. We found no evidence of superior performance of the selected strain over the wild strains under current or predicted future climatic conditions of temperature, DO, and salinity. At least one wild population from the Oti River may possess the traits for superior performance under high temperature and low DO conditions. Further studies should concentrate on comparing the Sabare strain with the Akosombo strain under both lab and pond conditions and increase experimental replications to confirm the suggested differences and the heritability of those performance traits for selective breeding.

ACKNOWLEDGEMENTS

We are grateful to the following individuals of KNUST and Virginia Tech who assisted with the field and laboratory studies: Nathaniel Gyasi Adjei, Abigail Ebachie Tarchie, Anthony Aliebe, Rapheal Nsiah-Gyambibi, Emmanuel Nyamekye, Iris Fynn, Yaw B. Ansah and Kwasi Obirikorang. We also thank Richmond Bulley and Umar Farouk, formally of University of Development Studies, Nyankpala campus, who served as field and laboratory technicians and Virginia Tech students Stephen Floyd, Tim Lane, and Vinnie Siegel for their assistance with the genetic analysis. Finally, we would like to sincerely thank Mr. Francis Adjei, Pilot Aquaculture Centre manager, Fisheries Commisison, for his dedication to this project and for providing the Akosombo strain of *O. niloticus* for the laboratory growth experiment and genetic analysis. We thank the contingent of local fishermen who assisted with fish and water quality sampling at Aframso, Sabare, and Binaba throughout the study.

LITERATURE CITED

Al-Asgah, N.A., and A. Ali, 1997. Growth performance and body composition of *Oreochromis niloticus* reared at different water temperatures. Annales De Zootechnie 46: 331-338.

Azaza, M.S., M.N. Dhraief, et al. 2008. Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. Journal of Thermal Biology 33: 98-105.

Baras, E., B. Jacobs, et al., 2001. Effect of water temperature on survival, growth and phenotypic sex of mixed (XX-XY) progenies of Nile tilapia *Oreochromis niloticus*. Aquaculture 192: 187-199.

Charo-Karisa, H., H. Komen, et al. 2006. Genetic and environmental factors affecting growth of Nile tilapia (*Oreochromis niloticus*) juveniles: Modelling spatial correlations between hapas. Aquaculture 255: 586-596.

Cnaani, A., E.M. Hallerman, M. Ron, J.I. Weller, Y. Kashi, G.A.E. Gall, and G. Hulata. 2003. A chromosomal region with quantitative trait loci affecting cold tolerance and fish size in an F_2 tilapia hybrid. Aquaculture 223: 117-128.

Dewedar, R., 2013. Fast-growing fish variety could benefit Egypt and West Africa. The Science and Development Network. http://www.scidev.net/en/agriculture-and- environment/fisheries/news/fast-growing-fish-variety-could-benefit-egypt-and-west-africa-.html (Retrieved 4/24/2013).

Drummond, C.D., L.D. Solis Murgas, et al. 2009. Growth and survival of tilapia *Oreochromis niloticus* (Linnaeus, 1758) submitted to different temperatures during the process of sex reversal. Ciencia E Agrotecnologia 33: 895-902.

El-Sayed, A.-F.M., 2006. Tilapia culture in salt water: environmental requirements, nutritional implications, and economic potentials. En: Editors Suarez, E.L.C. et al. Advances en Nutricion Acuicola VIII. Symposium Internacional de Nutricion Acuicola: 15-17. November. Universidad Autonoma de Nuevo Leon, Monterey, Nuevo Leon, Mexico.

El-Sayed, A.-F.M., and M. Kawanna. 2008. Optimum water temperature boosts the growth performance of Nile tilapia *Oreochromis niloticus* fry reared in a recycling system. Aquaculture Research 39: 670-672.

El-Zaeem, S.Y., M.M.M. Ahmed, et al. 2011. Production of salinity tolerant Nile tilapia, *Oreochromis niloticus* through traditional and modern breeding methods: II. Application of genetically modified breeding by introducing foreign DNA into fish gonads. African Journal of Biotechnology 10: 684-695.

Ficke, A.D., C.A. Myrick, et al. 2007. Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries 17: 581-613.

Handisyde, N.T., L.G. Ross, et al. 2005. The effects of climate change on world aquaculture: A global perspective. DFID, London, UK.

Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

Kamal, H.A.M.M., and G.C. Mair, 2005. Salinity tolerance in superior genotypes of tilapia, *Oreochromis niloticus*, *Oreochromis mossambicus* and their hybrids. Aquaculture 247: 189-201.

Kolding, J., L. Haug, et al., 2008. Effect of ambient oxygen on growth and reproduction in Nile tilapia *Oreochromis niloticus*. Canadian Journal of Fisheries and Aquatic Sciences 65: 1413-1424.

Leung, T.L.F., and A.E. Bates, 2013. More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security. Journal of Applied Ecology 50: 215-222.

Likongwe, J. S., T. D. Stecko, et al., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). Aquaculture 146: 37-46.

Pandit, N.P., and M. Nakamura, 2010. Effect of high temperature on survival, growth, and feed conversion ratio of Nile tilapia, *Oreochromis niloticus*. Our Nature 8: 219-224.

Papoutsoglou, S.E., and G. Tziha, 1996. Blue tilapia *Oreochromis aureus* growth rate in relation to dissolved oxygen concentration under recirculated water conditions. Aquacultural Engineering 15: 181-192.

Place, A.R., 1983. Isozymes. Current Topics in Biological and Medical Research 10(Genetics and Evolution): 147-170.

Paugy, D., C. Leveque, and G.G. Teugels. 2003. The fresh and brackish water fishes of West Africa, Volume II. IRD Editions, Publications Scientifiques du Museum, MRAC., Belgium.

Rengmark, A. H., and A. Slettan, et al., 2007. Identification and mapping of genes associated with salt tolerance in tilapia. Journal of Fish Biology 71: 409-422.

Schofield, P.J., M.S. Peterson, et al., 2011. Survival, growth and reproduction of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). I. Physiological capabilities in various temperatures and salinities. Marine and Freshwater Research 62: 439-449.

Streelman, J.T., and T.D. Kocher, 2002. Microsatellite variation associated with prolactin expression and growth of salt-challenged tilapia. Physiological Genomics 9(1): 1-4.

Tran-Duy, A., J. W. Schrama, et al., 2008. Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*. Aquaculture 275: 152-162.

Williams, L., and A. Rota, 2010. Impact of climate change on fisheries and aquaculture in the developing world and opportunities for adaptation. International Fund for Agricultural Development, Rome, Italy.

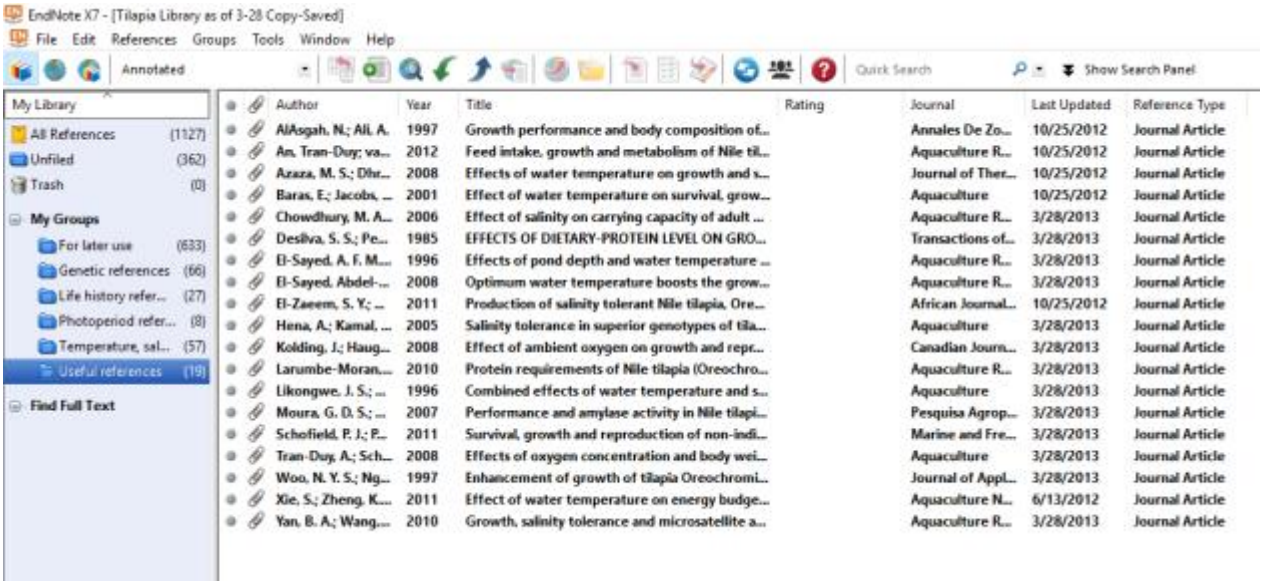
Xie, S., and K. Zheng et al., 2011. Effect of water temperature on energy budget of Nile tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 17: E683-E690.

Yan, B.A., and Z.H. Wang, 2010. Growth, salinity tolerance and microsatellite analysis of the F-2 reciprocal hybrids of *Oreochromis niloticus* x *Sarotherodon galilaeus* at different salinities. Aquaculture Research 41: e336-e344.

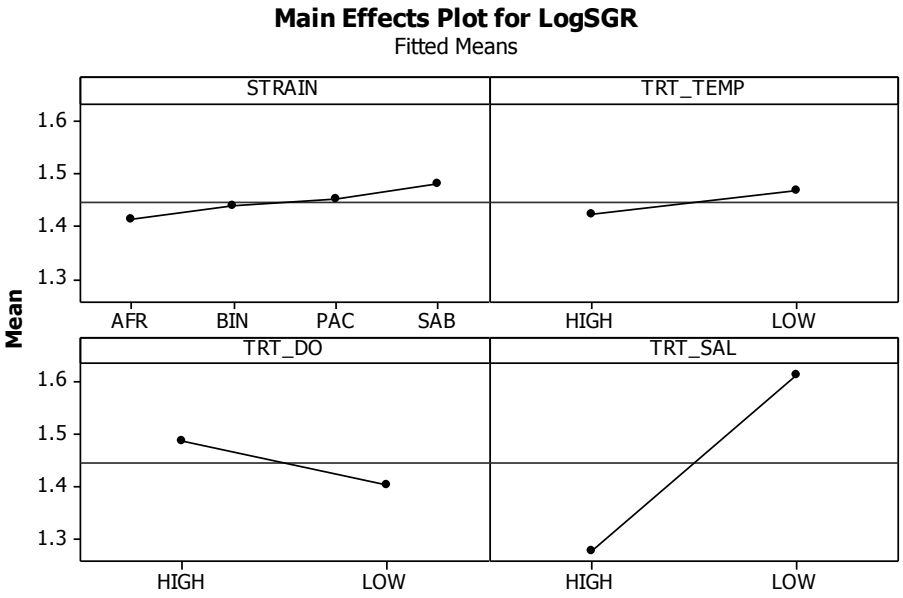
Zambrano, L., and E. Martinez-Meyer et al. 2006. Invasive potential of common carp *Cyprinus carpio* and Nile tilapia *Oreochromis niloticus* in American freshwater systems. Canadian Journal of Fisheries and Aquatic Sciences 63: 1903-1910.

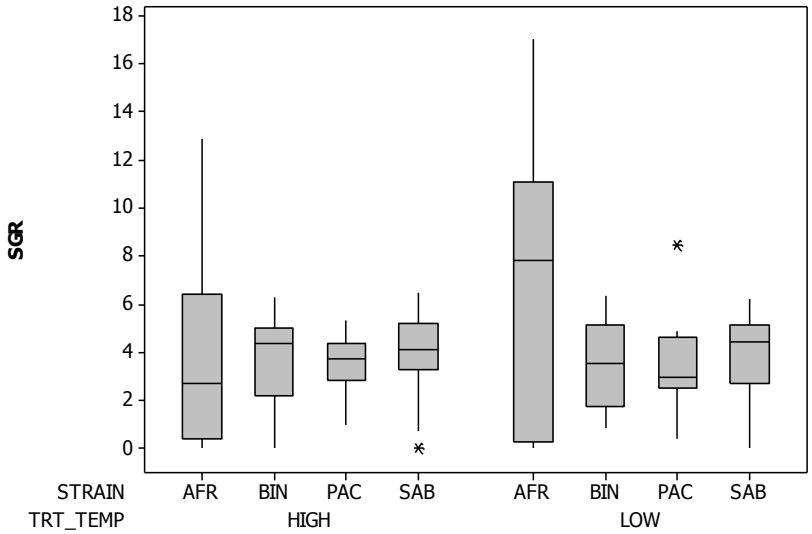
APPENDICES

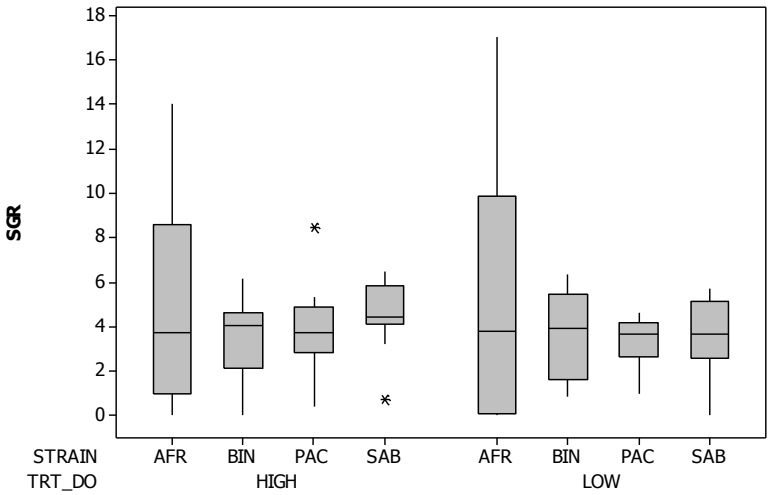
Appendix A. List of papers used in the meta-analysis, in EndNote library.

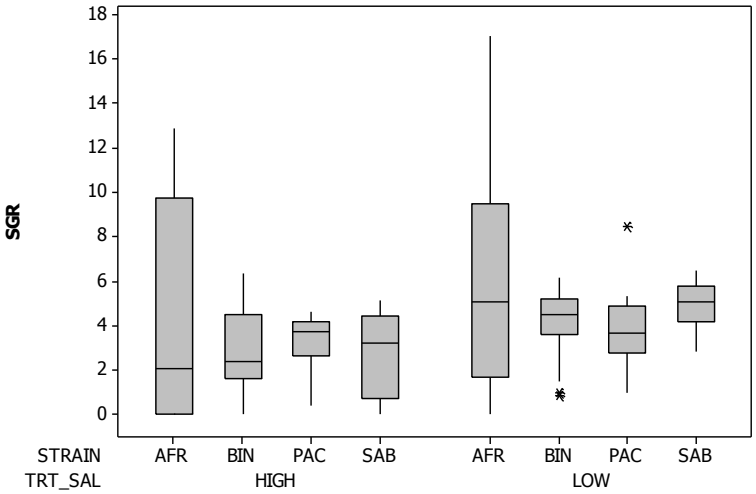


Appendix B. Additional results for lab experiments.



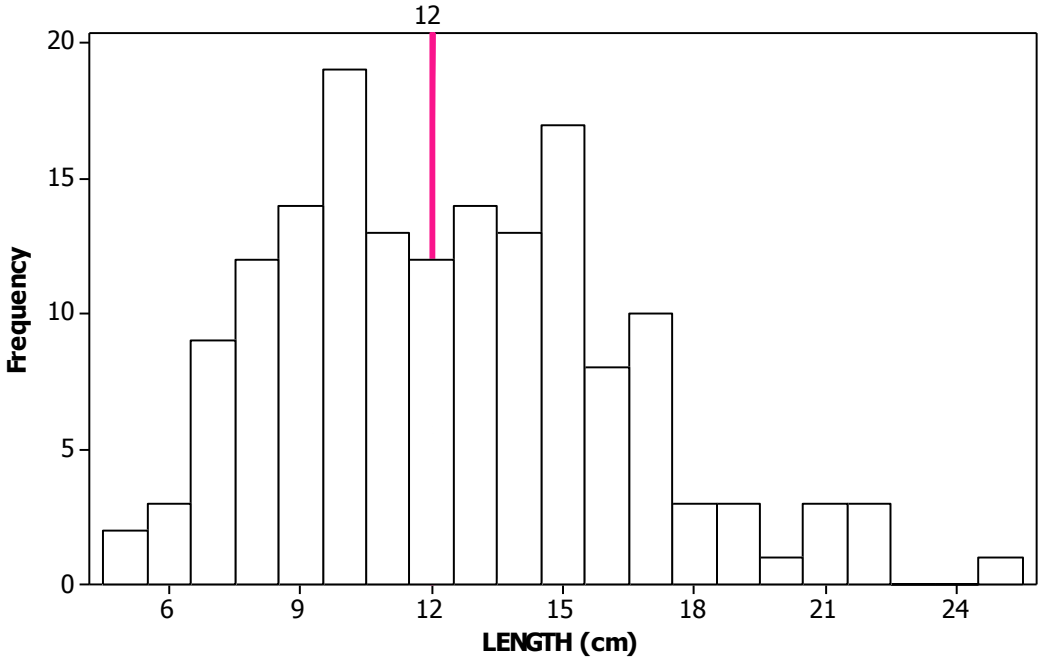

SGR
18
16
14
12
10
8
6
4
2
0
STRAIN
AFR
BIN
PAC
SAB
AFR
BIN
PAC
SAB
TRT_TEMP
HIGH
LOW


SGR
18
16
14
12
10
8
6
4
2
0
STRAIN
AFR
BIN
PAC
SAB
AFR
BIN
PAC
SAB
TRT_DO
HIGH
LOW

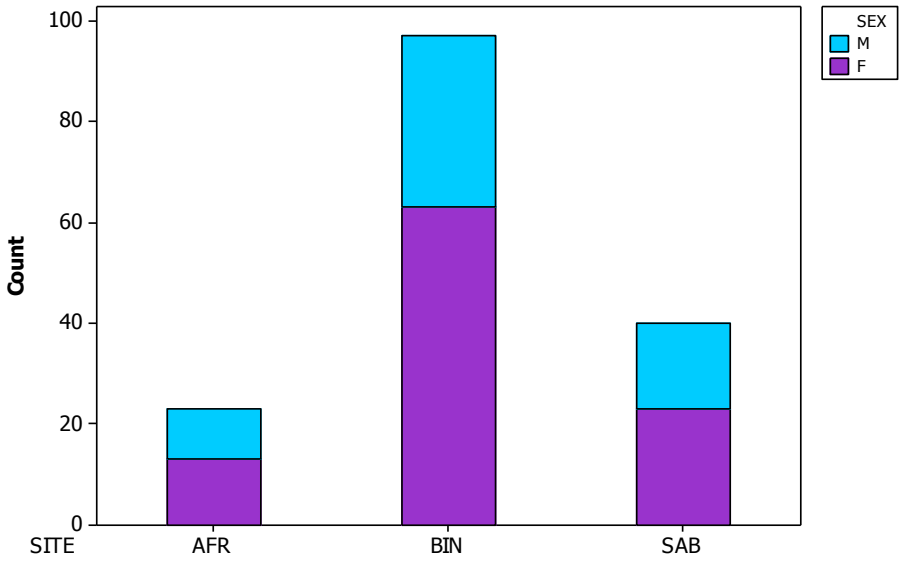

SGR
18
16
14
12
10
8
6
4
2
0
STRAIN
AFR
BIN
PAC
SAB
AFR
BIN
PAC
SAB
TRT_SAL
HIGH
LOW

Appendix C. Additional life-history results.

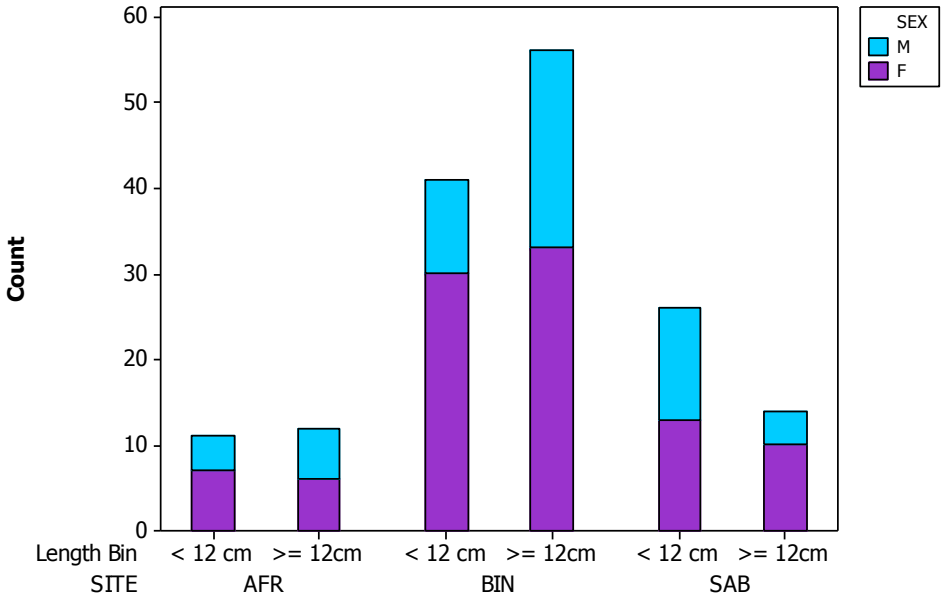
(i). Length distribution of preserved specimen sexually identified, N = 160



(ii.) Sex Ratios



(iii.) Sex ratio by site & size



Appendix D. Additional genetics results

(i). Numbers of alleles per locus per population.

Locus	Afram	W. Volta	Akosombo	Oti	Mean	s.d.
UNH130	5	4	5	4	4.500	0.577
UNH180	7	6	7	9	7.250	1.258
UNH858	15	20	11	15	15.250	3.686
UNH925	14	16	11	14	13.750	2.062
UNH934	8	8	4	6	6.500	1.915
Mean	9.800	10.800	7.600	9.600	9.450	1.340
s.d.	4.438	6.870	3.286	4.827	4.856	1.494

(ii). Expected heterozygosity per locus per population.

Locus	Afram	W. Volta	Akosombo	Oti	Mean	s.d.
UNH130	0.65706	0.67541	0.58701	0.60254	0.63051	0.04241
UNH180	0.70960	0.61263	0.74802	0.72429	0.69864	0.05948
UNH858	0.92316	0.93598	0.90226	0.86497	0.90659	0.03103
UNH925	0.89353	0.86072	0.87175	0.86025	0.87156	0.01558
UNH934	0.73277	0.60046	0.21751	0.57401	0.53119	0.22035
Mean	0.78323	0.73704	0.66531	0.72521	0.72770	0.04854
s.d.	0.11793	0.15232	0.27940	0.13755	0.17180	0.07310

(iii). Analysis of Molecular Variance (AMOVA) design and results.

Source of variation	d.f.	Sum of squares	Variance components[1]	Percentage of variation
Among groups	3	32.589	0.08574 V_a	4.47
Among populations within groups	1	4.682	0.04305 V_b	2.25
Among individuals within populations	144	308.555	0.35493 V_c	18.52
Within individuals	149	213.500	1.43289 V_d	74.76
Total	297	559.326	1.91660	

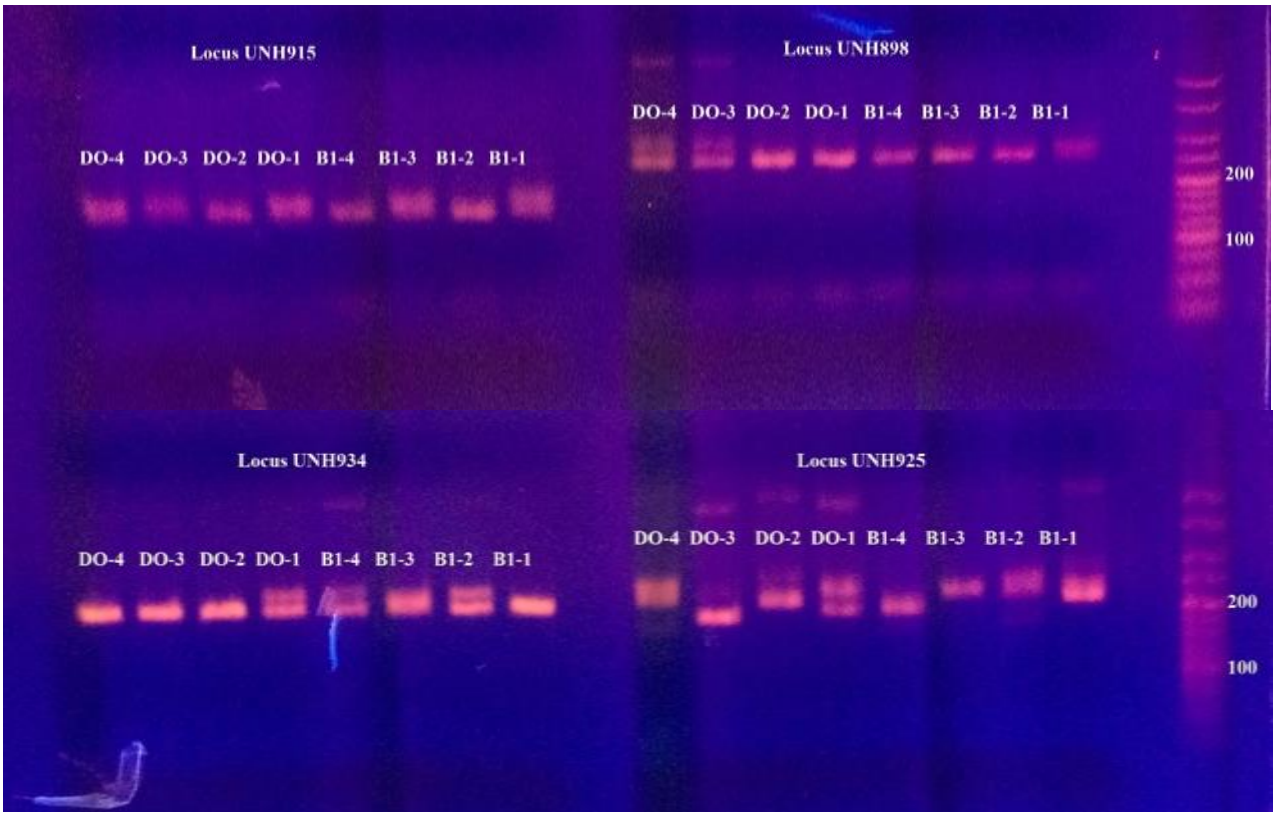
---

[1] Variance components with different superscripts are significantly different
at the p =0.05 level.

(iv). Population[1] pairwise F_{ST} values.

	1	2	3	4
1	0.00000			
2	0.04893	0.00000		
3	0.08288	0.07963	0.00000	
4	0.02682	0.05364	0.11770	0.00000

1 Populations: 1-Afram River, 2-White Volta River, 3-Akosombo strain, 4-Oti River.



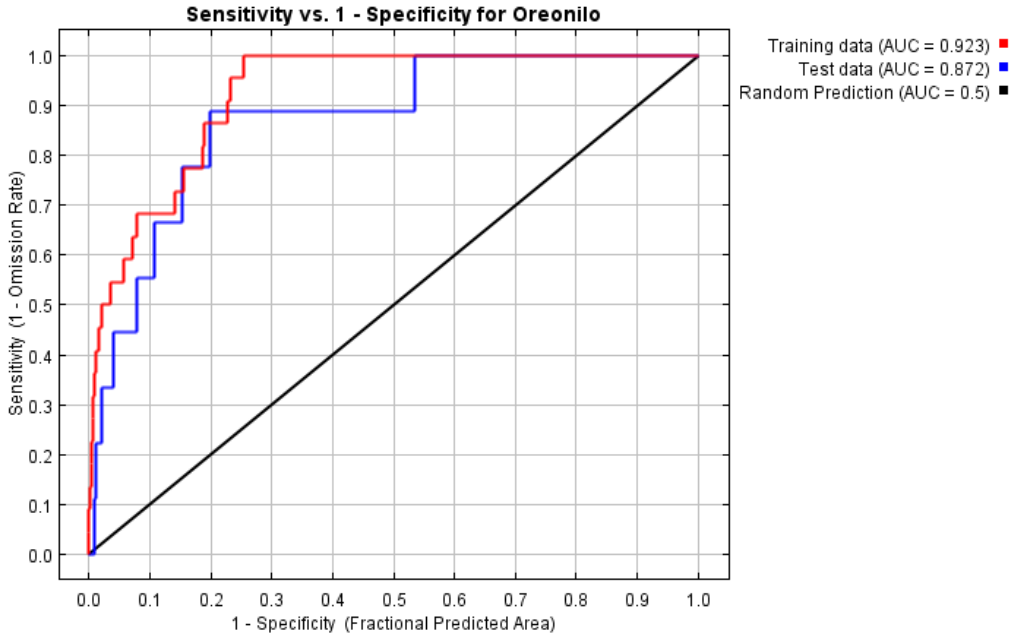
PCR products of individuals from Akosombo (DO) and Binaba (B1) populations at four microsatellite loci. Product size (base pairs) averaged around 200 bp; labels were added to ladder (right) for reference. After initial testing, loci UNH898 and UNH915 showed little to no variation and were excluded from future analyses. Both UNH925 and UNH934 exhibited moderate variation and were selected for DNA fragment analysis.

Appendix E. Additional methods and results of the Nile tilapia distribution models.

Definition of bioclimatic variables used in the models

BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (BIO2/BIO7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter

Additional plots and results from the MaxEnt tilapia distribution model



Analysis of variable contributions

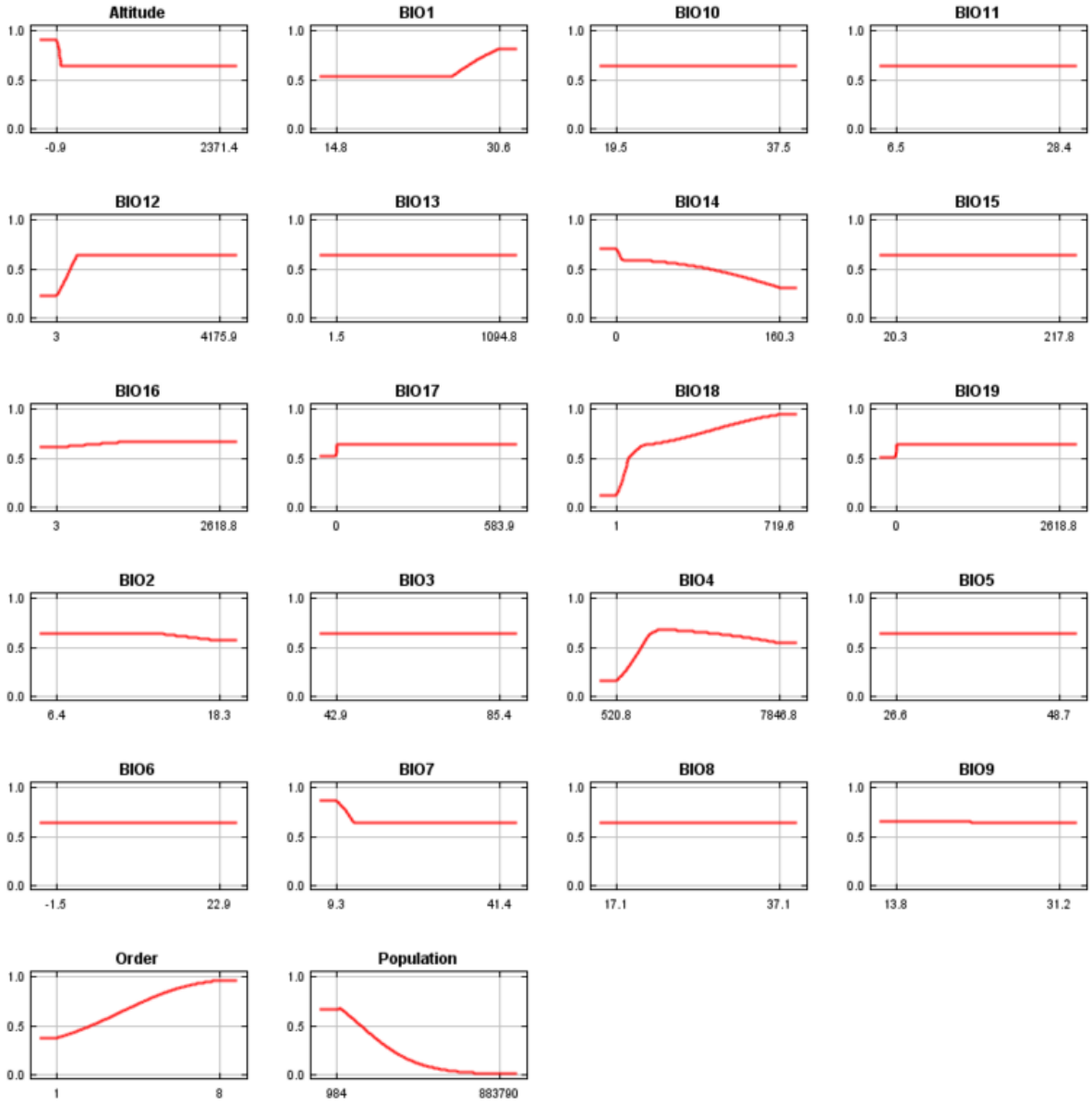
The following table gives estimates of relative contributions of the environmental variables to the Maxent model. To determine the first estimate, in each iteration of the training algorithm, the increase in regularized gain is added to the contribution of the corresponding variable, or subtracted from it if the change to the absolute value of lambda is negative. For the second estimate, for each environmental variable in turn, the values of that variable on training presence and background data are randomly permuted. The model is re-evaluated on the permuted data, and the

resulting drop in training AUC is shown in the table, normalized to percentages. As with the variable jackknife, variable contributions should be interpreted with caution when the predictor variables are correlated.

Variable	Percent contribution	Permutation importance
Order	48.9	18
BIO12	19.3	20.5
BIO18	14.9	37.3
BIO14	3.7	0.1
BIO11	3.3	0
Altitude	3	5.1
BIO4	2.1	14.1
Population	2	0
BIO19	1.1	0
BIO17	0.6	0
BIO1	0.6	2.3
BIO7	0.5	2.3
BIO2	0.1	0.2
BIO16	0	0
BIO9	0	0.2
BIO3	0	0
BIO5	0	0
BIO15	0	0
BIO6	0	0
BIO13	0	0
BIO8	0	0
BIO10	0	0

Partial response curves

These curves show how each environmental variable affects the MaxEnt prediction. The curves show how the logistic prediction changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. Note that these represent ‘partial’ or ‘marginal’ plots correcting for the effect of all other variables in the model. In other words, the curves show the marginal effect of changing exactly one variable, whereas the model may take advantage of sets of variables changing together.


Altitude
1.0
0.5
0.0
-0.9
2371.4
BIO1
14.8
30.6
BIO10
19.5
37.5
BIO11
6.5
28.4
BIO12
3
4175.9
BIO13
1.5
1094.8
BIO14
0
160.3
BIO15
20.3
217.8
BIO16
3
2618.8
BIO17
0
583.9
BIO18
1
719.6
BIO19
0
2618.8
BIO2
6.4
18.3
BIO3
42.9
85.4
BIO4
520.8
7846.8
BIO5
26.6
48.7
BIO6
-1.5
22.9
BIO7
9.3
41.4
BIO8
17.1
37.1
BIO9
13.8
31.2
Order
1
8
Population
984
883790